

CYTOTOLOGY NOTES

Zoo 125

(Zoo 226)

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1942

See Fig. 6, p. 23 Wilson. The cell, general view.

Required Lab-work:

4 drawings Golgi

8 drawings chromosomes

in Prog. of genesis.

Technique references:

Lee 137 X

Bowen Ann Rec 38 (1938)

Romeis '32 Taschenbuch

Grindia guineensis

adults, but is difficult.

Margantia sp. will be
seen up. Other sp.:

Nyera - large green bug, found on
elephant trees with berries

Cochliotus thistles

Bacchynema fruit trees

Anasa squash

Orthoptera: Dissosteira

Mus.

Do not anesthetize
Dissect and fix immediately

Alexander (1932). In vital staining at lower pH there is a suppression of granule formation, a staining of the nucleus and a diffuse staining of the cytoplasm. The stain effects are probably through C₄ changes as pH is lowered at low nH.

Wilson, Introduction
Sharp pp.

Cytology - history

Early work in embryology. - Wolf, Malpighi

1838 Schleiden and Schwann. The cell is the unit of structure of the organism. Formed by de novo crystallization.
Mol, Nägeli botanical aspects cell division
Reinke, Vielkow 1852-55 omnia cellula e cellula

Modern Cytology, the basis of contemporary work, lies in
1880-1905: van Beneden, the Hertwigs, Boveri, Wilson

The basic question in most fields has not really been answered but perhaps reformulated.

Theory of Fertilization

Light as an abnormal factor. a rather cautious, cynical attitude to general fundamental Theoratology; the key to life not well justified.

Strangeways &anti QMS 71

Belan ZIAV (1928)

The early cell lineage studies on Salpa, etc., were a lot of work, but only descriptive, not expository. Schleiden didn't

work on fixed dead cells. There are various tricks. Vital staining: - some abnormality introduced by dye.

Usually cytoplasmic components are stained. Under conditions of high pH nucleic acids may stain somewhat in chromatin

Even unstained cells must be examined cautiously. Sperm cells are much more visible. Observations have repeatedly been made on sperm cells. In tissue culture, optical conditions are never favorable.

It is amazing that anything comes out of fixation techniques

Chromosomes are easier to technique than chondroosomes.
Chondroosomes may never be globular.

The generalized picture of the cell — o. Wilson

All substances must pass through membrane; in some cases, only a plasma membrane; in others a definite cell membrane may be torn off.

Golgi usually in relation with central body.

In plants, no central body is present, but the spindle and chondroosomes act as if one were present.

Chromatin is defined as the material constituting the chromosomes, usually Feulgen positive.

Ground substance of the cell

residues of fibrillae, etc. ?

With development of immersion lenses, in the '80s activity began on the fundamental structure of cytoplasm. In living cells this is not clear, but there are appearances in fixed material.

Sigif. Science 73 1931

Micday, Science 84 1936

Bendix, Anat Rec 72 1938

Banga & Spath-Gyorgyi, Sc 92 1940

Pollister, Physiol Rev 14 1941

Wilson '24 pp 57-78

Symposium on the Structure

of Protoplasm 1942.

Sigif. "Protoplasm"

Fibrillae: Husky cytoplasm complicated terminology.

A. Reticular (v. Beneden) network or weave

B. Filar (Flem, Heiden.) discontinuous

Acicular Betschli, Wilson microsomes $> 2 \mu$.

microsomes. Living eggs seemed foam like. Hypothecum seemed enceloid. Also in some fish pupations.

But In annelid and echinoderm egg are found yolk, pigment granules, etc., which are not particularly

essential. True chloroplasts always $\leq 2\mu$, and under certain technical conditions anything might be identified with them.

granule defined as something angular.

Granular The ultimate unit is a granule, the smaller mesoebioblasts (Altmaier) many of which are now recognized as mitochondria. Continuous phase right?

Schmitz has seen these divided and concluded: none granula granulae. The bioblasts hypothetically are the same as Baerin's pycnocytes and Weismann's karyophores. When Altmaier, even before his work was discounted.

V. before.

Wilson Ann. Natur. 60

Interest has been renewed. ideas such as structural protein, etc. and long molecules. The diluted structure protein is steady... (long molecules).

Picken.
See also: Pfeffer. The Fine Structure of Biological Systems. in
(Proceedings of Roy. Soc.).

Biol Rev 15:133 (1940)

Bowen. J. Cellule 39:123 (1929)

Pfeffer indicates mitochondrial orientation parallel to direction of streaming and active. This is believed to be due to the orientation of long molecules and the exclusion of mitochondria from the lines of flow. There are similar relations of chromosomes and dictyosomes even in plant cells where the asters are not visible.

Schmidt. Protoplasma Monogr. II (1937)

Bipartition phenomena studied; indicate orientation in asters, spindles, (chromosomes?) No orientation outside the asters.

Early considerations are superseded. New attack on a molecular level

General cytology considers the standard equipment of the cell:

- Nucleus
- Centrioles
- Golgi
- Mitochondria
- (Plastids)

10/7/42

Wilson '24 670-700

Centriole

Heidenhain Plasmazellen I

Bowen J. Morph 39:351 1924

Johnson 2. Weiss 2001 140:115 1931

Pollister Biol Bull 65:521 1933

Fay Biol Bull 54, 56, 63, 65

" Anat Rec 46, 56

The centriole is frequently found at the astral center of the cell. It is most readily seen at metaphase, where the astral-spindle poles point it out.

In 1887 von Breden and Bovis recognized its independent existence. The whole region is called a centrosome (Wilson, p. 673.) The poleparts of the spindle and aster may stain more heavily and obscure the centriole. It is the centriole, the distinguishing mark being small granules at the center, that is the morphologically important and persistent structure. The centrosome is merely the confluence of astral rays.

The superficially obvious function of the centriole is as the spindle regulator in mitosis.

J

see Heidenhain's

← Rabl - all cells have 2 centrioles. ∴ the function in non-dividing cells is as an organizing center.

In epithelial cells, polarity is frequently determined by the position of centrioles.

If the centriole has generally constant positions, may it not have a general function?

(Similar to Golgi, see Baranowski 47:261 1938)

(John 1877) If epithelial cells reverse their polarity, the centriole moves, as in the enamel organ.

Between 1890 and 1910... a large literature on centrioles.

In epithelial cells, the distal centriole frequently bears a flagellum.

10/9/42

Centrioles: characterized by position, form, staining.

Benda Arch Anat Phys (Phys) 1901 Stain techniques are not too reliable.

Remy Zeitsch. Anat. 73: 338 1924 Feulgen negative (carothers?)

Walter Anat Rec 42 ✓ 1929 But perpetuation of cell and components usually is concerned with nucleic acids being present.

Kinoshita Anat Rec 34 ✓ 1927 later with nucleic acids being present.

May be protein; probably not lipid

In intestinal epithelium, stains like cement substance, which is probably polysaccharide.

Still open question.

? proved functions:

1. Organogenesis. By helices with multiple centrioles, each develops an aster for the second division. Aster are rays of oriented protein molecules.

2. Blepharoplasty. Induces flagellum formation. Unflagellate cells rather widespread. Sperm flagellum is best known.

Bonnef J Morph 39: 351 (1924...) Diverse centriole history in spermatogenesis, after and previous ones.

Third maturation. Starts growth at Telophase II, thus divides into p and d. The history of d₁ and d₂ does not yet seem rationalizable. Most commonly d₂ forms a ring. Possibly the distal are not entirely homologous.

Seminiferous tubules by Huettnner,
Schade.

Huettnner Z. Zellf. 19: 119

Henneberg Arch Anat Phys I ('98)

10/4 Blypharoplast can act as division center.

Henneguy & Tinkossek claim any vibratile processes arise from centrole; thus basal bodies of ciliace homologous with centroles. Can a centrole multiply?

corollary: can a ciliated cell divide?

Jordan claims ciliated cells divide amitotically. Presumed that before ciliogenesis, basal granules can be found.

But Benda, Grunder, Weber unmistakably showed mitosis in ciliated cell.

v. Mihalko 134, ovulet epithelium. Appearance of flagella in "subepithelial" cells, still attached to granules. These may be leucocytes and degenerate epithelial cells, instead of a progressive process.

Pollister doubts that basal granules are products of ~~cilia differentiation~~; back are centroles, multiplication put as products of the cilium, which is not necessarily homologous with a flagellum.

Can centroles multiply autonomously?

Boveri denied de novo formations.

Huettenrauch traced them through mitosis in Drosophila eggs. Becker in leucocytes; Vallentyne in Salpa; Pollister... The case for genetic continuity is clear. But occasionally de novo formations do occur: as in the spermatogonial divisions of *hydophyta* and *pterophyta*. In II, this centrole acts as a blypharoplast and multiflagellate mobile sperm are formed. Thus the centrole is not a self-perpetuating body, but the

Sturdivant J Morph 1934 Cleveland product of something which is, like the nucleus.
In *Acanthocephala* spermatoocytes, the centrole is intermediate.
In many protozoa, an aster is organized within the nucleus.

Pollister PNAS 25, " PNAS 39 10/16/42 Atypical spermatogenesis and meiosis: oligogenesis
sperm as in *Trochophora*, subcarinata, other pulmonates.
Perhaps because of physiological maturity or pre-
fertilization, some cells are abnormal —

Only 2 chromosomes segregate normally. In, with-
drawn,acentric degenerate, through peculiar vesicles. A
small nucleus with 2 chromosomes. At Anaphase II
1 chromosome to each pole. Therefore there are only 4 centri-
chromatids per quartet. A small nucleus is formed and
karyokinesis follows.

In atypical oocytes, there is one centriole, 1 pair
debris. As they break up they can be counted. They double
in number at Anaphase I. They are not entirely oppor-
tuned to the spermatid. Counts in Telophase II or
early spermatid are certain.

The extra centrioles comparable to theacentric
chromatids. Therefore, the supernumerary centrioles are
the accumulated centrioles.

This emphasizes the centrole - centriole relation-
ship. All good cases of centriolar division in somatic cells
occur at metaphase or later — after it has been in
relation to the chromosomes through the spindle.
In vivo pair, the centrole is Feulgen-negative.

"centrole is a material formed by the chromozone."
Other cases give strong evidence for nuclear origin: dye,
etc. In *Mesocilia*, the aster and centrole appear, as anti-

pated midlate anaphase, 3rd spermatogenesis division.

In cytoplasmic formations (which may be diffusion centers) centrioles may be present, but they do not occur before breakdown of germinal vesicle. v. Wilson.

Monasteries?

Schneider Biol Biull 70 1936

In Anopheline spermatocytes, a distinct granule is seen as the karyosome; stained very similarly to centriole. D'Addington proposes as nucleus on theoretical grounds.

10/21/42

Chondriosomes

Schultze (1861) recognized granulation in protoplasm.

Altmann bioblast theory, now known as mitochondria. Only granules are alive. Various shapes, says. Any stainable granule source is included. Not all self-duplicating; many secretory structures.

The development of techniques stimulated research.

Mitochondria have survived from the bioblast theory. Benda developed specific methods and differentiated mitochondria from other granules. Now an enormous literature.

2 older reviews:

Duisberg Arch Zellf 6 (1914) from other granules. Now an enormous literature.

Universal occurrence probable

Cowdry, Carnegie 271, 1918
(done later)

Nodes of uniform diameter; rounded ends. All similar in any one type of cell. Ought not to be really granular; may be misinterpreted as such. Very easily distorted by a poor fixator. Specific artifact studies.

Lewis & Lewis Gen. Cytology '24

Ranftzkyw Arch Zellf (1926)
(1923)

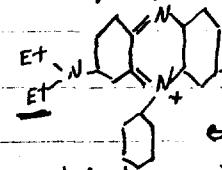
Shape of mitochondrion is reversibly affected by environment. Their orientation indicates a molecular basis.

Bansky & Geeth Anat Rec 57 (1933) structure of cytoplasm.

Michaelis Arch mikr Anat
Exhibit stud 55, 558

Specific stain: Janus Green B.

Cowdry Arch Anat 19:423 1916

 Cells very rapidly absorb Janus Green B into the cytoplasm. Mitochondria. They must absorb very highly since they are only .2 μ in diameter. Easily reduce the dye, anaerobically, through a rhodo and leuco form. The rhodo is diethyl safranine !! involving split molecule. The process is reversible!!!! Two methyls are specifically required, and the phenosafranine.

Functional aspects:

Symbiotic organisms: often more recently by Wallin, Porter. Many morphological resemblances to bacteria. Some instances of incipient bacterial symbioses now known. Alterations over geological time have led to present forms. They have never had any demonstrated significance.

Desirable organization in some scorpion spermatozoa indicates some function!

J.L.

Porter Les Symbiontes 1918
Wallin Symbioticaeum... 1927

Claude has reported submucic acid in granules from centrifuging, which may be mitochondria.

Claude CSH & B Sym. 9 (1911)

Pollester et al., ultraviolet studies indicate no different absorption at 2600 Å. Considerable refraction.

Nikolskine may not be 1923/41

chemically identical with mitochondrial precursors.

Chemistry: Lipid and protein components. The lipids stain in the differential stain technique.

Regaud: Rat testis. Precipitation; CrO₃ preserves them. Post-chrome required on basis of fat solubilities.

Regaud CRSB 6:718 (1908)

Faure-Fremiet, Schenckmer 11:457 (1910) Faure-Fremiet: similar results. Fat reaction in mitochondria found on cytolysis. Fat dissolved out; protein remains

Mayer, Rathen & Schaeffer

analogy with fats: only unsaturated lipoids absorbed. Bentley, J Path & Path 16:607 (1914) with dyes. Schaeffer Red is negative. Assume therefore

ibid

1929

a lipoprotein complex. Not determinable to be phospholipine. General statements to that effect may be erroneous. Various favorable evidences:

Bentley & Hoerr An Rec 60:444 '34
Bentley & Hoerr An Rec 69:341

37 Russo: Lecitin injections increase oocyte maturation. Löwsehins: Analogy with myelin structures in albumen, due to surface action. "phospholipid".

The melting point, density are higher than saturated fats in "UC" material. But protein condensator would account

for this.

Specificity?

Milson's test is positive.

Spermatozoa are rich in phosphatides - perhaps in sperm tail?

Bensley isolated "mitochondria" from liver cells, by slow centrifugation. Proximate analysis of mass:

40% fat 60% protein. dry weight

Detailed fat analyses: (1937)

Protein (and unknowns) 64.67 100-200 mg
sampled

Lipoid: 35.33

as: glycerides 28.88%

lecithin 4.2 %

Sterol 2.25%

Some X-ray studies indicate a periodic pattern in the mitochondria.

Claude: analysis of granules: 60% protein
40% lipid, largely phospholipin.

Bensley Science Oct 9, 1942.

Most recent: Bensley -

Lecithin 45-58% of the lipid content of liver "mitochondria". Therefore, there is appreciable phospholipin.

Mitochondrial Function:

Horning

Kochling J Morph

1. Enzymatic: 4 zinc dyed protolytic enzymes. (Actually all large molecules.)

MacIntosh Biol 17:851 '23

" dust Steg. Biol 3:233 '26, not this reflected. MacIntosh - but the specificity of Janus Green B is Robertson showed the leuco-form does not precipitate enzymes, while mitochondria will react in leuco form, if oxygen is later readmitted.

Cowdry Amer Nat 60:157 '26 The MacIntosh school has a lipid orientation function; the de Novey & ... Anat Rec 34:313 '27 mitochondria increase the surface (see Cowdry, de Novey).

The lipid acts as a semi-molecular solvent, and acts as an active surface for protoplasmic and excretory synthesis. (after Langmuir) Robertson has shown an increase in the Forêt-Savigne Pr. 6:84 ... rate of synthesis of proteins in tryptic solution, when lipid is added as emulsion.

Hirsch Z. Zellf. 13:37 (1926)

Duthie PRS B 114:20

de Novey & Cowdry: measured various surfaces of formed Bowen G. Rev Biol 4:488 1929 components of pancreas cells. Assume, hypothesis into - Bowen Z. Zellf. 9 1929 tried semipolar compounds on a granular adsorbent. see Paper.

Heredity factors?

Guillemin: Animal mitochondria homologous with plant plastids. Conceivably factors in cytoplasmic inheritance. Duration of self-penetration.

Hewes, Benda, ... conceived a morphogenetic function, but this was carried too far: the physiologically basis of all fibrosis. Now abandoned.

Hausch, Duthie: vital observations on pancreas cells.
 The earliest glycogen granules appear basally in the pancreas cell, at mitochondrial surfaces!
 May have some function in myogenesis.

10/30. Homologies in plants -

In the meristematic cells, thread-like bodies appear which may be proplastids. Stainably with Janus Green B. There are also "mitochondria" which do not become plastids.

Guillemond '39
The Cytoplasm of the Plant Cell.
 Guillemond distinguishes between active mitochondria, the chondriome, which become plastids, and inactive mitochondria which are homologous.

Bowen '39, thought they could distinguish them ^{by} and stain reactions.

Plastids contain ribonucleoprotein.

SPERMATOGENESIS

There is very great variation in spermatogonia. They are more species characteristic than any other cell, and perhaps most readily analyzable.

There are 4 constant morphological components, derived from:

nucleus → head ...

Acroblast (Golgi) → acrosome, "peracrosome," refringent granules.

Centriole - centriolar apparatus (flagellum).

Mitochondrial Apparatus ...

Primitively, flagellated, with head anterior.

Acrosome may be anterior, sometimes lateral (*Lepisma*) or even posterior. Usually very small, but in the hemipterae *Notonecta* it is very large. The dimensions of this spermatocyte:

Significance of the 5th layer (mitochondrial)
granules in fertilization:
cortical reaction ?? ?

Overall length = 1500 μ

See Bowes & Morph. 1922 nucleus = 200 μ

Studied in *Ascidia* Sp. metagenesis acrosome = 650 μ

Wilson tail = 650 μ

The acrosome is not a peracrosome in function !!

Some mitochondria are always present, always posterior to the nucleus. May grow down to form a middle piece or a spiral organ. [May contribute to skeletal rods in head.]

Significance is not clear. The symbiotacists would claim that the perinuclear fusion may be a synergy, or loss of identity. [Characteristic onion structure of membranous] These mitochondria probably do not participate in ova development of the egg.

Non-flagellate sperm. (see Bowen's review.)

Mitochondria in plant
spermatogenesis??

Relyea: series of pictures...

Koltyoff...

Fate of nucleolus in genuine sperm, best represented in mammals or lower vertebrates.

Mitochondria studies on sperm
material, after extracting
nucleoprotein??

Gatesley, Bowen

General features:

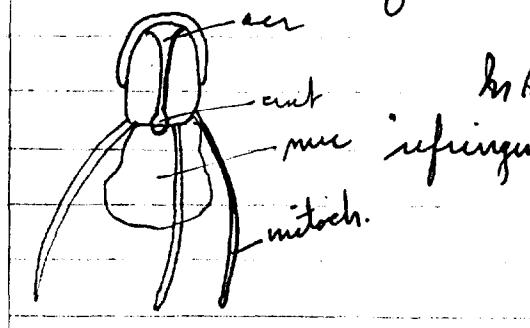
Acrosome from Golgi secretion; Golgi itself lost with cytoplasm. Formation variable in time and place.

Mitochondria free in spermatid

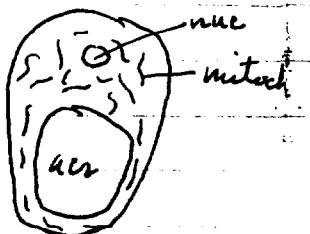
11/4/42

Worley, La Celleule 48 '39
Stanley J Morph '32
Bowen Aust Rec. 31 '25

Various Decapoda: only part of the capsule is homologous with the acrosome: the stainable ring at the anterior end of the capsule



In Arcanus, non-motile, the more refringent body is the Golgi derivative



Further notes on spermatogenesis